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Vascular inflammatory markers in early-onset obese and type 2 diabetes subjects before and after three months' aerobic exercise training

MENSUD HATUNIC, FRANCIS FINUCANE, NICOLE BURNS, DECLAN GASPARRO, JOHN J NOLAN

Abstract

Early-onset type 2 diabetes (T2DM) may lead to very early vascular complications. Cardiovascular mortality is two to five times higher in adults with diabetes than in people without diabetes. The cardiovascular risk of young people with T2DM is unknown. T2DM in young people is associated with marked visceral obesity, insulin resistance and microalbuminuria. We recently showed that these subjects did not improve in either fitness (maximum volume of oxygen consumption, VO_2max) or glucose disposal after exercise training.

Seven subjects with early-onset T2DM (aged 26.1 ± 0.9 years, body mass index [BMI] $35.6 \pm 1.2 \text{ kg/m}^2$) and 14 age-matched obese subjects with normal glucose tolerance (aged 25.6 ± 0.9 years, BMI $34.3 \pm 1.4 \text{ kg/m}^2$) underwent aerobic training for 12 weeks. Serum vascular inflammatory markers (high-sensitivity C-reactive protein [hsCRP], soluble intercellular adhesion molecule [sICAM-1], soluble vascular cell adhesion molecule [sVCAM-1], E-Selectin and P-Selectin) were measured before and after the training programme.

At baseline, plasma concentrations of vascular inflammatory markers were significantly elevated in both groups. They did not improve after exercise.

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Key words: cardiovascular risk, C-reactive protein, E-Selectin, exercise, intercellular adhesion molecule, obesity, P-Selectin, type 2 diabetes, vascular inflammatory markers, vascular cell adhesion molecule.

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Introduction

Patients with diabetes mellitus are at increased risk of developing cardiovascular disease. Previous studies have shown that the risk of cardiovascular mortality is two to three times higher in men with diabetes and three to five times higher in women with diabetes than in people without diabetes.^{1–4} The worldwide prevalence of diabetes in adults is expected to increase from 5% to 6.2% from 2003 to 2025.⁵ Until recently, type 2 diabetes was regarded as a disease that typically affected the middle-aged and elderly. Evidence is accumulating that the onset of type 2 diabetes in younger adults is increasing. Even children and adolescents are now part of the diabetes epidemic.⁶ Obesity has increased by 70% in adults aged 18–29 years, and type 2 diabetes has increased in parallel by 70% in adults aged 30–39 years over the last decade, making young adults the fastest-growing adult group for both obesity and type 2 diabetes.^{7,8}

Early-onset type 2 diabetes appears to be a more aggressive disease. We have recently reported that much younger subjects with type 2 diabetes are obese and severely insulin-resistant with markedly abnormal cardiovascular risk markers, similar to the findings in patients 30 years older.⁹ In fact, the population of young adults with type 2 diabetes has a more adverse risk profile for cardiovascular disease when compared with age-matched subjects without diabetes, but also relative to older patients with type 2 diabetes.^{9–11} If these findings are confirmed in other larger populations, they will have profound social and economic implications.

Individuals who report regular physical activity are less likely than sedentary individuals to die from coronary heart disease and stroke. Several studies have assessed the independent and combined effect of obesity and physical fitness on mortality.^{12–14} There is an abundance of evidence that exercise delays or prevents the development of type 2 diabetes in at-risk populations.^{15–18} Smaller studies have shown that exercise improves insulin sensitivity and glucose metabolism.^{19,20} We have demonstrated that short-term exercise training increases insulin sensitivity by more than 50% in obese middle-aged patients (mean age 45 years) with type 2 diabetes.²¹

However, in contrast to the middle-aged patients, we have recently shown that patients with early-onset (before age 25) obesity and type 2 diabetes are resistant to the expected metabolic benefits of aerobic exercise training.²²

Table 1. Clinical and anthropometric characteristics in young obese and diabetic subjects pre- and post-exercise

	Controls pre	Controls post	p value	YT2 pre	YT2 post	p value
N	14			7		
M:F ratio	4:10			3:4		
Mean age (years)	25.6±0.9			26.1±0.9		
Height (m)	1.69±0.03			1.73±0.05		
Weight (kg)	97.47±4.65	95.6±4.54	0.07	108.3±8.7	109.4±8.4	NS
BMI (kg/m ²)	34.25±1.44	33.58±1.34	NS	35.63±1.23	36.02±1.17	NS
Systolic BP (mmHg)	116.1±4	112.8±4	NS	119±4.69	121±4.15	NS
Diastolic BP (mmHg)	71.3±2.4	69.6±2.3	NS	77±2.5	81±2.3	NS
Waist:hip ratio	0.89±0.02	0.9±0.02	NS	0.99±0.05	0.96±0.03	NS
HbA _{1C} (%)	5.43±0.11	5.32±0.16	NS	8.46±0.6	8.41±0.73	NS
VO ₂ max (ml min ⁻¹ kg ⁻¹)	2.77±0.24	3.36±0.41	<0.0001	2.48±0.31	2.72±0.35	0.078

Values are presented as mean ± standard error of the mean in parentheses

Key: YT2 = young type 2 diabetes; BMI = body mass index; BP = blood pressure; NS = not significant; HbA_{1C} = glycosylated haemoglobin; M = male; F = female; VO₂max = maximum volume of oxygen consumption

Neither maximum volume of oxygen consumption (VO₂max) nor insulin sensitivity improved in the young diabetes subjects despite full compliance with three months of exercise training. VO₂max did improve (20% increase) in the non-diabetic obese subjects, but glucose disposal did not.²²

Plasma levels of soluble adhesion molecules have been studied in various inflammatory and pro-thrombotic disorders. An increase in soluble vascular cell adhesion molecule (VCAM) and soluble intercellular adhesion molecule (ICAM) has been reported in coronary artery disease (CAD).²³ An increase of soluble P-Selectin (sP-Selectin) has also been reported in arteriosclerosis.²⁴ Soluble E-Selectin (sE-Selectin) is increased in patients with early atherosclerosis or those with manifest atherosclerotic disease.²⁵ Evidence is accumulating that high-sensitivity C-reactive protein (hsCRP) is predictive of future coronary events.²⁶

The aim of this study was to compare circulating levels of cardiovascular inflammatory markers (hsCRP, VCAM, ICAM, sP-Selectin, sE-Selectin) in the previously described young obese and young T2DM subjects at baseline and after 12 weeks of supervised aerobic exercise intervention. We hypothesised that, even without whole body changes in VO₂max and glucose uptake, which might require a more prolonged or varied intervention, exercise would lead to an improvement in a variety of the surrogate markers of cardiovascular risk in these subjects.

Methods

Patients aged between 15 and 30 years with obesity or with type 2 diabetes were recruited from the outpatient clinics at St James's Hospital, as previously described.²² Subjects with co-existing illnesses or secondary forms of diabetes were excluded from the study. The local Research Ethics Committee approved the protocol and written informed consent was obtained.

Subjects attended the Metabolic Research Unit for test-

ing at 8 am for initial investigations. A full history and routine fasting blood samples were taken. Diabetes was excluded in the obese group by a standard 75 g oral glucose tolerance test. Each subject with diabetes was confirmed negative for glutamic acid decarboxylase antibodies. Waist:hip ratio, weight, height and body mass index (BMI) were measured. Blood pressure was measured using the left arm after the subject had been sitting comfortably for five minutes, using an oscillometric device (Omron® 705 CP). Three readings were taken and the lowest one recorded. Body composition was assessed using an electrical impedance device (Tanita® Body Composition Analyser). Urinalysis and 12-lead ECG were also performed.

VO₂max was measured in an exercise laboratory, under the supervision of an exercise physiologist. The test involved progressively increasing the workload on a bicycle ergometer to the subject's maximal ability, in a stepwise fashion. Heart rate and blood pressure were monitored during the test.

Following successful screening, the training protocol consisted of 60 minutes of aerobic exercise four days a week for 12 weeks at 70% of each subject's maximum oxygen uptake on a bicycle ergometer or treadmill.²² Subjects had their blood pressure and heart rate monitored at each training session throughout the period of the study. The subjects maintained a stable diet and treatment for diabetes during the period of the exercise programme. All measurements were repeated after 12 weeks of the programme.

The vascular inflammatory markers were measured in frozen fasting serum collected and stored at -80°C. Serum concentration of hsCRP was determined by immunonephelometric assay (Immulite® 2000); normal values were < 5 mg/L. sICAM-1, sVCAM-1, sE-Selectin and sP-Selectin levels were assessed with an enzyme-linked immunosorbent assay using monoclonal antibodies specific for each of those adhesion molecules (R&D Systems, Abingdon, Oxfordshire,

Table 2. Comparison markers of vascular inflammation in young obese controls and T2DM subjects pre- and post-exercise

	YO pre-exercise	YO post-exercise	p value	T2DM pre-exercise	T2DM post-exercise	p value
HsCRP (mg/L)	6.3±1.7	7.1±2.3	0.3	5.9±2.6	7.2±2.6	1.0
E- Selectin (ng/ml)	72 (40–85)	61 (37–85)	0.3	63 (43–89)	52 (43–64)	0.2
P- Selectin (ng/ml)	115 (91–143)	122 (100–133)	0.7	127 (98–147)	130 (80–148)	0.9
VCAM (ng/ml)	526 (440–655)	541 (429–685)	0.9	667 (528–789)	668 (560–1026)	0.8
ICAM (ng/ml)	252 (205–297)	256 (213–334)	0.9	316 (285–377)	292 (270–375)	0.3

Values are presented as mean ± standard error of mean or the median (interquartile ranges) in parentheses

Key: YO = young obese; T2DM = type 2 diabetes; hsCRP = high-sensitivity C-reactive protein; VCAM = vascular cell adhesion molecule; ICAM = intercellular adhesion molecule

UK). Normal reference range values: for sICAM 115–306 ng/ml, sVCAM 379–991 ng/ml, sE-Selectin 29.1–63.4 ng/ml, sP-Selectin, 51–113 ng/ml.

Statistical analysis

Data were expressed as mean ± standard error of mean or the median (interquartile ranges 25–75%). Differences between groups were analysed by a Student's paired *t*-test or unpaired *t*-test. Because of the skewed distribution of soluble adhesion molecules, differences in concentration were evaluated by non-parametric statistical procedures (Mann-Whitney U). Wilcoxon Signed Rank Test was performed to examine differences before and after exercise. A *p*-value < 0.05 was considered to be statistically significant. SPSS for Windows 12.0 was used for statistical analysis.

Results

Patient characteristics are outlined in table 1. Both groups were matched for age and BMI. There were no significant differences between the groups at baseline in VO₂max, systolic or diastolic BP. The T2DM group had a significantly higher glycosylated haemoglobin (HbA_{1c}) level and waist to hip ratio. After completion of the exercise programme, VO₂max increased by 24% in the obese non-diabetic control group, but did not increase in the group with T2DM. Nor were there any significant improvements in anthropometrics or insulin sensitivity, measured with the hyperinsulinaemic glucose clamp technique, as we recently reported.²²

Measurements of vascular inflammatory markers are outlined in table 2. There were no significant differences between the groups for any of the markers, either at baseline or after exercise intervention.

In both groups, the concentrations of hsCRP, sE-Selectin and sP-Selectin were markedly elevated at baseline, and did not change after three months of supervised aerobic exercise intervention. In both groups, the concentrations of sVCAM and sICAM were in the high normal range at baseline and did not change after exercise.

Discussion

This study has shown that concentrations of a range of cardiovascular inflammatory markers (hsCRP, sP-Selectin, sE-

Selectin) in young obese and young T2DM subjects are elevated at baseline and do not change significantly after 12 weeks of supervised aerobic exercise. The values of sVCAM and sICAM were in the high normal range and were not significantly different after the exercise programme.

It is known that diabetes mellitus and obesity confer higher risk of cardiovascular disease and represent an important global public health problem.^{1–5} In our previous studies we confirmed that our young patients with T2DM are obese, severely insulin-resistant and have a more adverse cardiovascular risk profile than older patients.^{9,10} Because early-onset type 2 diabetes is a relatively new clinical phenomenon, it is not clear which factors (such as severe obesity, insulin resistance or low-grade inflammation) are responsible for this adverse risk profile. There are as yet no long-term studies that describe the natural history of diabetes and its complications in these subjects. Early-onset visceral obesity and severe insulin resistance are probable contributors to the inflammatory cardiovascular risk milieu and to vascular stiffness, but this remains to be confirmed.

We hypothesised that aerobic exercise training would improve insulin sensitivity, physical fitness and a range of the associated phenotypic abnormalities in this very insulin-resistant population. Although VO₂max increased by 20% in the obese control group, there was no significant improvement in the group with diabetes. Nor was there any improvement in whole body glucose uptake in either group, as recently reported.²² These patients have a markedly adverse cardiovascular risk profile that did not improve with short-term exercise training. Surprisingly, the surrogate markers of cardiovascular risk were not improved by this three-month intervention. Thus, it is possible that the underlying cellular abnormalities conferring severe insulin resistance and exercise resistance in these patients may overlap with those contributing to risk of early cardiovascular disease. One likely shared mechanism could be the effects of lipotoxicity in both the target tissues for insulin action, particularly skeletal muscle and liver, and in the cardiovascular system. These patients are clearly dyslipidaemic at baseline, and remain so after aerobic exercise intervention.²² Lipotoxicity may ultimately be exerted through impairment of mitochondrial function,²⁷ another cellular mechanism potentially linking

the resistance to exercise in skeletal muscle and the effects on the cardiovascular system. Low-grade inflammation is another potential mechanism integrating these abnormalities. Whether inflammation *per se* represents a modifiable risk factor in obesity is currently uncertain, although recent studies have suggested that some common preventive therapies, such as the use of statins, may reduce inflammatory markers.²⁸ None of the patients in the current study were receiving statin therapy, nor is it current practice to administer statins to patients in this age group with type 2 diabetes.

While the current results are disappointing, additional studies will clearly be needed to investigate the role of dose, intensity and duration of exercise and other concurrent interventions in the modification of both insulin resistance and adverse cardiovascular risk in high-risk young patients with type 2 diabetes and severe insulin resistance.

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Conflicts of interest statement

None declared.

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